

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE  
APPLICATION FOR PATENT

**FLASK AND METHOD FOR DRYING BIOLOGICAL MATERIALS**

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Background of the Invention

1. Field of the Invention

10 The present invention is related to a flask or container which is preferably used for drying biological materials. More specifically, embodiments of the present invention provide a flask for receiving biological materials, and method for treating or processing the biological materials while contained in the flask.

15 2. Statement Regarding Federal Sponsored Research and Development

Embodiments of this invention were made with Government support under Grant No. 6600100C8048, awarded by the Department of Defense Advanced Research Projects Agency (DARPA). The Government has certain rights to embodiments of this invention.

20 3. Description of the Prior Art

Dried biological materials are becoming increasingly useful in agriculture, biotechnology and medicine. For instance, freeze-dried human blood products, vaccines and the like are already in use, or are proposed to be in use, in clinical settings for both animal and human applications. In the field of biotechnology, biosensors have wide spread applications. In all such cases, long term storage under sterile conditions, and often under unfavorable environmental circumstances, is a requirement.

25 Conventional devices in the market do not optimally provide drying of biological materials under sterile conditions, while allowing for certain desired contamination-free processing of the biological materials under defined conditions, such as during drying, storage and re-hydration. More particularly, because biological materials show much improved survival

if they are exposed to water vapor prior to immersion in liquid water, it is desired to pre-hydrate biological samples with water vapor without contaminating the biological samples.

A patentability investigation was conducted to determine the state of the art with respect to solving problems of contamination while processing biological materials during drying, storage and re-hydration, and the following U.S. Patents were discovered: U.S. Patent No. 4,232,453 to Edelmann; U.S. Patent No. 4,275,511 to Parkinsen, et al.; U.S. Patent No. 4,966,469 to Fraser, et al.; U.S. Patent No. 5,154,007 to Piunno, et al.; U.S. Patent No. 5,689,595 to Sutherland, et al.; and U.S. Patent No. 6,122,836 to Tenedini, et al.

U.S. Patent No. 4,232,453 to Edelmann discloses a tray for holding a biological specimen and a submersible container for freeze-drying the specimen. A heating element is taught for melting a synthetic resin for embedding the specimen therein.

U.S. Patent No. 4,275,511 to Parkinsen, et al. discloses an evaporator/sublimator flask having a straight sided cylinder, preferably made of borosilicate glass tubing of sufficient wall thickness to prevent implosion when subjected to a high vacuum. The straight sided cylinder is open at one end and sealed at the other. An elastomer cap is disposed over the open end of the cylinder.

U.S. Patent No. 4,966,469 to Fraser, et al. discloses a flask for freeze-drying. A positioning device engages the top of the flask and comprises a generally circular stopper having an opening. An annular tube extends through the stopper and into the flask. A thermocouple is coiled around the lower part of the annular tube.

U.S. Patent No. 5,154,007 to Piunno, et al. discloses an apparatus and describes a method for distillation drying of one or more biological samples. The apparatus includes a retaining assembly, a vacuum assembly, a cooling assembly, a monitoring assembly and a control assembly for actively regulating the temperature and pressure conditions of biological tissue so that biological samples may be dried without damage.

U.S. Patent No. 5,689,895 to Sutherland, et al. discloses a device for positioning a probe (e.g., a temperature sensor) in a flask for freeze-drying. The device includes a stopper secured to an open end of the flask. The stopper has a center opening and at least one radial opening spaced from the center opening. The radial opening allows for fluid communication between inside and outside of the flask when the stopper is secured to the open end of the flask. The center opening receives a guide tube which extends into the flask and receives the probe.

U.S. Patent No. 6,122,836 to Tenedini, et al. discloses a freeze-drying apparatus and associated lyophilization procedures employing vapor flow detection and/or vacuum control for monitoring and control of a lyophilization process. The vapor flow detector (e.g., a windmill sensor) is disposed to monitor vapor flow from product undergoing lyophilization.

5       None of the foregoing patents teach a flask, device or container which permits drying (freeze-drying, air-drying, foam drying) of biological materials under sterile conditions and which allows for processing under defined conditions during drying, storage and re-hydration. Therefore, what is needed and what has been invented is a flask and method which overcomes the contamination deficiencies of the prior art. What is more specifically needed and what has  
10       been invented is a flask for drying (e.g., freeze-drying) substances under sterile conditions, and method for processing a substance under sterile conditions, including drying, storage, and re-hydration. In the method for processing, the flask is placed on a shelf of a freeze-drying and re-hydration apparatus where substances contained in the flask are freeze-dried and re-hydrated without contamination.

### Summary of the Invention

Embodiments of the present invention broadly provide a device for holding substances during drying. The device includes a flask having a structure defining an opening, a first filter member disposed in the opening, and a second filter member disposed in the opening juxtaposedly to the first filter member. The first filter member comprises at least one aperture sized to preclude the passing of bacteria there through. Preferably, first filter member comprises a plurality of apertures having an average opening with an average maximum dimension (e.g. diameter, maximum diagonal distance across opening, etc.) ranging from about 0.10  $\mu\text{m}$  to about 0.22  $\mu\text{m}$ . In one embodiment of the invention the first filter member has a higher flexibility than the second filter member. In another embodiment of the invention, the difference in average permeability or average maximum dimension of openings (i.e., the openings that permit gases or liquids to pass through) between the first and second filter members ranges from about 0.00  $\mu\text{m}$  to about 0.90  $\mu\text{m}$ . Thus, if one filter member has an average aperture opening of about 0.22  $\mu\text{m}$ , the other filter member may have an average aperture opening ranging from about 0.60  $\mu\text{m}$  to about 0.90  $\mu\text{m}$ . A retainer ring is engaged to the flask for retaining the first and second filter members in the opening. The structure of the flask additionally comprises a second opening wherein a third filter member maybe disposed. A temperature-conductive member passes through a side of the flask. The device or flask may be disposed in a freeze-drying apparatus where substances contained in the device are processed by freeze-drying and prehydration.

Embodiments of the present invention also provide a method for processing a substance under sterile conditions comprising disposing a substance in a flask, positioning the flask in a drying apparatus, and passing a drying medium through a first filter member and through a second filter member juxtaposed to the first filter member for drying the substance. The method may additionally comprise contacting the substance with a temperature-conductive member for monitoring the temperature of the substance. The temperature-conductive member typically passes through a side of the flask and has a thermocouple coupled thereto. The flask may be exposed to water vapor as desired for prehydration purposes.

These provisions together with the various ancillary provisions and features which will become apparent to those skilled in the art as the following description proceeds, are attained by

the methods and flask(s) of the present invention, preferred embodiments thereof being shown with reference to the accompanying drawings, by way of example only, wherein:

Variable	Unit	Mean	St. Dev.	Min.	Max.
Age	Years	45.1	10.2	25	65
Gender	Male/Female	0.52	0.50	0	1
Marital Status	Married/Single	0.68	0.48	0	1
Education	Years	12.5	2.1	8	16
Income	\$1000s	28.5	15.2	10	60
Home Value	\$1000s	115.2	45.8	40	200
Number of Children	Children	1.8	1.2	0	4
Health Insurance	Yes/No	0.95	0.23	0	1
Auto Insurance	Yes/No	0.98	0.13	0	1
Life Insurance	Yes/No	0.72	0.45	0	1
Property Tax	\$1000s	3.2	0.8	2	4
Crime Rate	Per 1000	12.5	3.2	8	18
School Quality	Score	78.5	5.2	70	85
Unemployment	%	5.2	1.5	3	8
Population Density	Per Sq. Mile	1200	300	800	1800
Climate Index	Index	65	10	50	80
Proximity to Water	Miles	15.2	8.5	0	30
Historical Landmarks	Count	2.5	1.5	0	5
Local Government Type	City/Town/Village	0.45	0.50	0	1
Local Economy Type	Manufacturing/Service	0.60	0.49	0	1
Local Industry Diversity	Index	45	15	30	60
Local Infrastructure Score	Score	85	5	80	90
Local Environmental Quality	Score	75	10	65	85
Local Cultural Diversity	Index	55	12	40	70
Local Healthcare Access	Score	90	3	85	95
Local Public Safety	Score	88	4	83	93
Local Economic Stability	Score	70	10	60	80
Local Social Cohesion	Score	82	8	75	90
Local Political Engagement	Score	78	12	65	90
Local Environmental Awareness	Score	85	10	75	95
Local Cultural Heritage	Score	72	15	60	85
Local Economic Resilience	Score	80	12	70	90
Local Social Inequality	Score	65	10	55	75
Local Political Corruption	Score	55	15	40	70
Local Environmental Degradation	Score	45	12	35	55
Local Cultural Homogeneity	Score	60	10	50	70
Local Economic Inequality	Score	50	15	40	60
Local Social Mobility	Score	70	12	60	80
Local Political Participation	Score	80	10	70	90
Local Environmental Policy	Score	85	8	80	90
Local Cultural Integration	Score	75	10	65	85
Local Economic Growth	Score	88	5	83	93
Local Social Justice	Score	78	12	68	90
Local Political Transparency	Score	82	10	72	92
Local Environmental Protection	Score	87	7	82	92
Local Cultural Preservation	Score	73	12	63	83
Local Economic Stability Index	Index	60	10	50	70
Local Social Cohesion Index	Index	75	12	65	85
Local Political Engagement Index	Index	80	10	70	90
Local Environmental Quality Index	Index	85	8	80	90
Local Cultural Diversity Index	Index	65	15	55	75
Local Economic Resilience Index	Index	70	12	60	80
Local Social Inequality Index	Index	55	10	45	65
Local Political Corruption Index	Index	45	12	35	55
Local Environmental Degradation Index	Index	35	10	25	45
Local Cultural Homogeneity Index	Index	50	12	40	60
Local Economic Inequality Index	Index	40	15	30	50
Local Social Mobility Index	Index	60	10	50	70
Local Political Participation Index	Index	70	12	60	80
Local Environmental Policy Index	Index	80	8	75	85
Local Cultural Integration Index	Index	70	10	60	80
Local Economic Growth Index	Index	85	5	80	90
Local Social Justice Index	Index	75	12	65	85
Local Political Transparency Index	Index	82	10	72	92
Local Environmental Protection Index	Index	87	7	82	92
Local Cultural Preservation Index	Index	73	12	63	83

### Brief Description of the Drawings

Fig. 1 is a top plan view of the device or flask for holding biological materials;

Fig. 2A is a side elevational view of the flask of Fig. 1 illustrating the filter cover and  
5 filter cap removed therefrom;

Fig. 2B is a front elevational view of the neck taken in direction of the arrows and along the plane of line 2B-2B in Fig. 2A;

Fig. 3 is an enlarged side elevational partial sectional view of the flask illustrating the top aperture with the filters and covering removed therefrom;

Fig. 4 is a top plan view of the flask illustrating the thermocouple probes which are to  
10 pass through a side of the flask and contact any substance therein;

Fig. 5 is a front elevational of the removable filter cap having a partially exposed filter;

Fig. 6 is an enlarged partial vertical sectional view of the two superimposed filters disposed over the flask opening with the cover or retainer ring coupled to the top of the flask such as to keep the two filters sandwiched over the flask opening;

Fig. 7 is the view of Fig. 6 with the filter cover in place and represented by dashed lines;

Fig. 8 is a vertical sectional view of the filter cover;

Fig. 9 is a partial perspective view of a freeze-drying apparatus containing the flask for treating or processing any biological materials contained in the flask;

Fig. 10 is a graph of freeze-drying sample temperatures during three simultaneous freeze-drying runs; and  
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Fig. 11 is an image of dried cells for the Example taken on film using an inverted microscope, showing the dried cells encased within strands of the drying matrix.

### Detailed Description of Preferred Embodiments of the Invention

Referring in detail now to the drawings for various embodiments of the invention, there is seen a flask, generally illustrated as **10**, having a bottom **12**, a rear wall **14** and side walls **16** and **18** bound to the bottom **12**, and a front wall assembly **20** bound to the bottom **12** and the side walls **16** and **18**. A top **22** is secured to the rear wall **14**, to the side walls **16** and **18**, and to the front wall assembly **20**. The flask **10** may be manufactured from any suitable material, preferably a transparent plastic (polyethylene, polypropylene, polystyrene, etc.).

The top **22** of the flask **10** includes an opening **30** with a perimeter **32**. Filters **34** and **36** are superimposedly disposed over the opening **30** such that the respective perimeters **34a** and **36a** associated with filters **34** and **36** extend beyond the perimeter **32** of opening **30** for structural support purposes, as best shown in Figs. 6 and 7. A cover or retainer ring **40** is conveniently coupled to the top **22** for holding the filters **34** and **36** in place superimposedly. The retainer ring **40** has an inwardly protruding lip **42** for defining an internal opening **44** to expose filters **34** and **36** and for protruding or lapping over the perimetrical fringes of filters **34** and **36** for posturing same in their filtering position, as best shown in Figs. 6 and 7. A removable filter cap **50** may be conveniently disposed within the ring **40** to cover internal opening **44** and superimposed filters **34** and **36** (see Fig. 7). As best shown in Fig. 8, filter cap **50** includes a continuous bottom **54** for immediately covering opening **44** and the filters **34** and **36**. Bottom **54** terminates in shoulder **58** and an upstanding wall **60** which forms an opening **62** to expose the bottom **54**. A depending ridge **64** is integrally secured to shoulder **58** for resting on, and being supported by, protruding lip **42** when the bottom **54** spacedly covers the superimposed filters **34** and **36**. The ridge **64** allows the bottom **54** to be spaced or separated from filter **36**.

The front wall assembly **20** is formed with a protruding hollow neck **70** to provide an opening **74** (see Fig. 2B) for placing and removing material **80** inside the flask. Material **80** may be any desired substance to be processed, such as biological materials. Removably secured to neck **70** is a cap **86**, preferably a cap **86** including an exposable filter **88** (see hydrophobic filter in Fig. 5) upon suitable rotation or manipulation. The cap **86** may be any suitable cap which is capable of being removably disposed around the neck **70**. In one preferred embodiment of the invention, the cap **86** is that manufactured under the trade name "Nunc EasYFlask closure" by Nalge Nunc International Corp. of Naperville, Illinois. This particular cap **86** is available as a

filter cap (with hydrophobic filter 88) or as a vent/close cap. The Nunc EasYFlask closure system of Nalge Nunc International Corp. allows one to open or close the flask 10 by merely a 1/3rd turn of the cap 86. Once one removes the cap 86, the opening 74 of the angled neck 70 allows easy access to the entire growth surface of the flask 10 with both pipettes and cell scrapers. The slope and the design of the neck 70 allow complete drainage, and yet minimal risk of any medium within the flask 10 splashing into the opening 74 of the neck 70 when disposing the flask 10 horizontally.

In a preferred embodiment of the invention, one or more temperature probes 90 maybe disposed such as to be in contact with substance or material 80 (see Fig. 1). Preferably, the probes 90 pass through side of side wall 16 and couple to thermocouple conductors 94-94 which extend to an indicator (not shown) for displaying temperature indicia of the substance or material 80. The temperature of the substance or material 80 is preferably monitored during processing, especially the drying process. The progress of the temperature of the material 80 during freeze-drying, air drying, or foam drying provides valuable information about the progress of treatment or processing, especially drying. For this purpose, the temperature probes 90 are placed in contact with the sample or material 80. The thermocouple conductor 94 is coupled to the probe 90 to monitor sample temperature.

Filter 34 is preferably a bacteria-filtering filter which precludes the entry of bacteria into the flask 10. Filter 36 is a prefilter, preferably for filtering large foreign particles, such as dust. Filter 36 is preferably a support membrane type filter which increases the structural rigidity of the combination of the superimposed filters 34 and 36. In operation, filter 34 would typically move or flex toward and/or against filter 36. Preferably, filter 34 has a higher flexibility than filter 36. Preferably further, filter 34 comprises a plurality of apertures having an average opening with an average maximum dimension (e.g. diameter, maximum diagonal distance across the opening, etc.) ranging from about 0.10  $\mu\text{m}$  to about 0.65  $\mu\text{m}$ , more preferably from about 0.10  $\mu\text{m}$  to about 0.45  $\mu\text{m}$ , and most preferably from about 0.10  $\mu\text{m}$  to about 0.22  $\mu\text{m}$ . Filter 36 comprises a plurality of apertures having an average opening with an average maximum dimension (e.g. diameter, maximum diagonal distance across the opening, etc.) ranging from about 0.60  $\mu\text{m}$  to about 1.0  $\mu\text{m}$ , more preferably from about 0.65  $\mu\text{m}$  to about 0.95  $\mu\text{m}$ , and most preferably from about 0.70  $\mu\text{m}$  to about 0.90  $\mu\text{m}$ . Stated alternatively, and in another embodiment of the invention, the difference in average size of filter openings between the filters



34 and 36 ranges from about 0.00  $\mu\text{m}$  to about 0.90  $\mu\text{m}$ , more preferably from about 0.45  $\mu\text{m}$  to about 0.75  $\mu\text{m}$ , and most preferably from about 0.50  $\mu\text{m}$  to about 0.70  $\mu\text{m}$ . Thus, if the average size openings in filter 34 is about .22 microns, the average size openings in filter 36 may range anywhere from about 0.60  $\mu\text{m}$  to about 0.90  $\mu\text{m}$ . Filters 34 and 36 may be manufactured from any suitable material, such as polyvinylidene fluoride, cellulose, fiberglass, etc. Preferably filter 34 is manufactured of polyvinylidene fluoride, while filter 36 is manufactured from fiberglass.

In a method for processing a substance or the material 80 under sterile conditions, the material 80 is disposed in the flask 10 via the opening 74 in the neck 70. The flask 10 containing the material 80 is then placed in a suitable apparatus for processing the material 80 with the flask 10. The apparatus for processing, generally illustrated as 100 in Fig. 9, may be any suitable apparatus for drying and/or rehydrating the material 80. A suitable apparatus 100 is that which is manufactured by Kinetics Group, Inc., and sold under the trade name FTS Systems Lyostar.

After the flask 10 containing the material 80 has been suitably disposed in apparatus 100, the material 80 is frozen within the flask 10 at a rate that is considered optimal for the sample and as can be controlled by an apparatus, such as apparatus 100. Once frozen to low temperature, such as  $-60^{\circ}\text{C}$  for example, the sample can be exposed to a strong vacuum as produced by the drying apparatus 100. The applied vacuum draws water out of the samples by sublimation of the frozen water, making the ice change directly into liquid vapor. The water vapor leaves the material 80 and passes serially through the filters 34 and 36 and is collected within the apparatus 100 by a condensor. This drying continues as the material 80 is slowly heated back to ambient room temperature under vacuum.

After freeze-drying, the flask 10 containing the freeze-dried sample may be removed from the apparatus 100 and for storage purposes. Storing in a sterile manner may be at room temperature in a suitable dry location while the freeze-dried material 80 remains in the flask. During storage, the filter cap 50 may be placed within the confines of the ring 40 to cover internal opening 44 of the retainer ring 40 and the superimposed filters 34 and 36. When it is desired to use material 80, the flask 10 including the material 80 is removed from storage and is subsequently disposed within a suitable humid chamber for rehydration of the freeze-dried material 80. During rehydration, minute particulates of water vapor pass through filters 36 and 34 while contamination is prevented from entering the inside of flask 10 by the filters 34 and 36. The water vapor is typically at a temperature ranging from about  $20^{\circ}\text{C}$  to about  $37^{\circ}\text{C}$ . The

remaining rehydration is then achieved by the addition of water, and/or a preferred resuspension solution, such as a cell growth medium, via the opening 74 in neck 70 of the flask 10.

Embodiments of the present invention will be illustrated by the following set forth example which is being given to set forth the presently known best mode and by way of illustration only and not by way of any limitation. All parameters such as concentrations, mixing proportions, temperatures, rates, compounds, etc., submitted in these examples are not to be construed to unduly limit the scope of the invention.

### EXAMPLE

The flask 10 may be loaded with a sterile sample, in a sterile hood, sealed, and then transferred to the drying apparatus (a freeze-dryer) 100, such as that manufactured by Kinetics Group, Inc. The sterile sample may be 293H human embryonic kidney cell line in 2.5ml buffer solution. When freeze-drying is complete, the flask 10 containing the freeze-dried sample may be stored without precautions against contamination since the flask 10 is a closed system. Vapor phase re-hydration may be accomplished simply by exposing the flask 10 to water vapor within a humidified incubator. Since the water vapor contacts the sample by passage through the bacterial filter 34, there is no risk of contamination. It is preferable to monitor sample temperature during the drying process. The progress of sample temperature during freeze drying, air drying, or foam drying provides valuable information about the progress of drying. For this purpose, the end of the temperature probe 90 may be placed in contact with the sample or material 80. The thermocouple wire 94 is coupled to temperature probes 90.

Material 80 used in the example consisted of a 2.5ml sample buffer solution containing 293H cells placed within flasks 10 via the opening 74 in neck 70. The flasks 10 were placed within the apparatus 100 for freeze-drying. The flasks 10 and samples 80 were frozen at 1°C per minute to -60°C and held at that temperature for 1 hour. The vacuum was initiated during this time interval while the samples were held at -60°C. As the vacuum was applied by apparatus 100, the change in sample temperature was observed via port 90 due to the heat released by the material 80 during loss of water, as indicated by Figure 10. Next, under vacuum, the samples were held at -25°C for an additional 6 hours. Following this time interval, the samples were subsequently slowly heated back to room temperature of +22°C over an 8 hour period while still under vacuum. Figure 10 depicts the process in its entirety and displays flask temperatures as

monitored through temperature port 90 of each flask and shelf temperatures, both as monitored by apparatus 100. Fig. 10 more specifically shows sample temperatures during three simultaneous freeze-drying runs, illustrating repeatable processing. All came to minimal water contents simultaneously. Residual water content was  $\leq 3\%$  by weight. Maintenance of sterility was assessed during processing. Thus, starting with sterile sample materials 80, the flasks 10 were subjected to freeze-drying, followed by re-hydration in culture media, in a sterile hood. Over the following nine days sterility was assessed by microscopy and pH change. Based on these criteria, no contamination was evident. Fig. 11 is an image of the dried cells for this Example. The Fig. 11 image was taken on film using an inverted microscope and shows the dried cells encased within strands of the drying matrix.

Therefore, due to the inherent design, having a set of filters 34 and 36 covering a smaller area than the top surface 22 of the flask and having a flask 10 that is of rigid, transparent plastic, one can view a sample 80 disposed within the flask 10 (prior to or after drying) directly using a microscope (preferably an inverted type or by inversion of the flask 10 on a standard scope) without any risk of contamination to a sterile sample 80. In other words, by having the filters 34 and 36 only covering a portion of the upper surface 22, there is a free visual path through the plane of the bottom surface 12 (via top surface 22) of the flask 10 for allowing microscopic (visible, fluorescence, etc.) viewing. With a microscopy, valuable photographs (micrographs, digital images, video, etc.) can be taken of the samples 80 during any stage of the process. These images can help one ascertain the quality of drying, the integrity of a sample, sample structure, and allow viability assays, while the samples 80 are still contained within the flask 10.

While the present invention has been described herein with reference to particular embodiments thereof, a latitude of modification, various changes and substitutions are intended in the foregoing disclosure, and it will be appreciated that in some instances some features of the invention will be employed without a corresponding use of other features without departing from the scope and spirit of the invention as set forth. Therefore, many modifications may be made to adapt a particular situation or material to the teachings of the invention without departing from the essential scope and spirit of the present invention. It is intended that the invention not be limited to the particular embodiment disclosed as the best mode contemplated for carrying out

this invention, but that the invention will include all embodiments and equivalents falling within the scope of the appended claims.

number	height	age	sex	hab.	species	fruit	stems
1121	1.5 m	20	M	forest	100%	young	young
1122	1.5 m	20	M	forest	100%	young	young
1123	1.5 m	20	M	forest	100%	young	young
1124	1.5 m	20	M	forest	100%	young	young
1125	1.5 m	20	M	forest	100%	young	young
1126	1.5 m	20	M	forest	100%	young	young
1127	1.5 m	20	M	forest	100%	young	young
1128	1.5 m	20	M	forest	100%	young	young
1129	1.5 m	20	M	forest	100%	young	young
1130	1.5 m	20	M	forest	100%	young	young
1131	1.5 m	20	M	forest	100%	young	young
1132	1.5 m	20	M	forest	100%	young	young
1133	1.5 m	20	M	forest	100%	young	young
1134	1.5 m	20	M	forest	100%	young	young
1135	1.5 m	20	M	forest	100%	young	young
1136	1.5 m	20	M	forest	100%	young	young
1137	1.5 m	20	M	forest	100%	young	young
1138	1.5 m	20	M	forest	100%	young	young
1139	1.5 m	20	M	forest	100%	young	young
1140	1.5 m	20	M	forest	100%	young	young
1141	1.5 m	20	M	forest	100%	young	young
1142	1.5 m	20	M	forest	100%	young	young
1143	1.5 m	20	M	forest	100%	young	young
1144	1.5 m	20	M	forest	100%	young	young
1145	1.5 m	20	M	forest	100%	young	young
1146	1.5 m	20	M	forest	100%	young	young
1147	1.5 m	20	M	forest	100%	young	young
1148	1.5 m	20	M	forest	100%	young	young
1149	1.5 m	20	M	forest	100%	young	young
1150	1.5 m	20	M	forest	100%	young	young
1151	1.5 m	20	M	forest	100%	young	young
1152	1.5 m	20	M	forest	100%	young	young
1153	1.5 m	20	M	forest	100%	young	young
1154	1.5 m	20	M	forest	100%	young	young
1155	1.5 m	20	M	forest	100%	young	young
1156	1.5 m	20	M	forest	100%	young	young
1157	1.5 m	20	M	forest	100%	young	young
1158	1.5 m	20	M	forest	100%	young	young
1159	1.5 m	20	M	forest	100%	young	young
1160	1.5 m	20	M	forest	100%	young	young
1161	1.5 m	20	M	forest	100%	young	young
1162	1.5 m	20	M	forest	100%	young	young
1163	1.5 m	20	M	forest	100%	young	young
1164	1.5 m	20	M	forest	100%	young	young
1165	1.5 m	20	M	forest	100%	young	young
1166	1.5 m	20	M	forest	100%	young	young
1167	1.5 m	20	M	forest	100%	young	young
1168	1.5 m	20	M	forest	100%	young	young
1169	1.5 m	20	M	forest	100%	young	young
1170	1.5 m	20	M	forest	100%	young	young
1171	1.5 m	20	M	forest	100%	young	young
1172	1.5 m	20	M	forest	100%	young	young
1173	1.5 m	20	M	forest	100%	young	young
1174	1.5 m	20	M	forest	100%	young	young
1175	1.5 m	20	M	forest	100%	young	young
1176	1.5 m	20	M	forest	100%	young	young
1177	1.5 m	20	M	forest			